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Chemical Characterization of Constituents Isolated from *Hamelia patens* and Investigating Its Cytotoxic Activity

M. A. I. Maamoun[1*], S. A. El-Sawi [1], H. M. Motawae [1], M. I. Fekry[2] and M. A. Abdel kawy[2]

[1]*Department of Pharmacognosy, National Research Centre, Cairo, Egypt.*
[2]*Department of Pharmacognosy, Faculty of Pharmacy, Cairo University, Cairo, Egypt.*

SRB cytotoxicity assay was performed for different extracts of *Hamelia patens* Jacq. family Rubiaceae; crude flowers (CF), crude leaves (CL), chloroform (Chl.L) and methanol (Me.L) fractions from leaves against liver (HepG-2) and breast (MCF-7) human carcinoma cell lines. Results demonstrated potent cytotoxic action against HEPG-2 for CF, Chl.L and Me.L with IC_{50} = 47, 30 and 44.4 µg/mL, respectively. On the other hand, CF, CL, Chl.L and Me.L had exerted powerful effect against MCF-7 with IC_{50} = 23.8, 25.5, 17.7 and 64 µg/mL, respectively. Chromatographic investigation of Me.L , resulted in the isolation and spectroscopic identification of rutin **(1)**, isoquercetin **(2)** and soyasaponin Bb **(3)**, which was isolated for the first time from the plant leaves and was identified by UPLC/ITMS/MS analysis. Comparative study for using ordinary method versus green methods of extraction was also inspected.

Keywords: Natural products, *Hamelia patens*, cytotoxicity, Soyasaponin Bb, UPLC\ITMS.

Introduction

Hamelia patens Jacq. is an ornamental plant belonging to family Rubiaceae. The most common chemical constituents in the family are monoterpene indole and oxindole alkaloids, triterpenes and poly hydroxylated phenolic compounds. The presence of these compounds in the family plants is considered as chemotaxonomic markers for these plants from different subfamilies and tribes [1]. Several phytochemical compounds were isolated and identified from the plant. From the aerial parts; palmirine, rumberine, hameline, pteropodine, isopteropodine, uncarine F and speciophylline oxindole alkaloids [2, 3], along with the monoterpenoid indole alkaloids tetrahydroalstonine and aricine [3], stigmasterol and ß-sitosterol [4]. Some phenolic compounds were detected by HPLC/ESI-MS analysis; hydroxycinnamic acid, catechin, caffeoylquinic acid, quercetin 3-O-rutinoside and kaempferol-3-O-rutinoside [5]. Many biological activities were reported for the plant; anti-bacterial [6], anthelmintic [7] and antifungal activities [8]. The current work aimed to explore the phytoconstituents from the plant cultivated in Egypt, isolating chemical compounds by chromatographic techniques and identification of these compounds by different analytical spectroscopic methods. Investigating the potential cytotoxic effect for crude extracts of flowers and leaves as well as the non polar and polar successive fractions of leaves was also carried out. Different extraction methods were used to discover the most dominant one that had given higher yield.

Experimental

Plant Material

Fresh aerial parts (leaves and flowers) of *H. patens* Jacq. were collected from the garden of National Research Centre, Dokki, Giza, Egypt and were kindly identified by the agricultural engineer Mrs. Trease Labib, consultant of plant taxonomy, Ministry of Agriculture and the ex-director of Al-Orman botanical Garden, Giza, Egypt and further confirmed by Dr. Mohamed El-Gebaly, Senior Botanist, National Research Centre, Egypt. The plant was dried in shade, ground and a voucher specimen was kept at Pharmacognosy department,

*Corresponding author e-mail: maimaamoun0nrc@gmail.com
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National Research Centre, Cairo, Egypt.

Plant Extraction

1) Extraction by cold percolation (CP)

100 g. of dried powdered *H. patens* leaves and flowers were, separately, soaked in stoppered containers with solvent 70% Methanol and allowed to stand at room temperature with frequent agitation. After 30 min (for comparison with other methods), the mixture was filtered. The process was repeated for six times, each time lasts for 3 days. The obtained samples were crude extracts of leaves (CL) and flowers (CF).

2) Microwave assisted extraction (MAE)

100 g. of dried powdered leaves and flowers were, separately, processed with 70% methanol in a microwave oven for 30 min. Another 100 g. of dried powdered leaves were successively extracted with chloroform then with methanol in microwave oven at the same conditions to yield leaves chloroform (Chl.L) and methanol fractions (Me.L).

3) Ultrasound-assisted extraction (UAE)

100 g. of dried powdered leaves and flowers were, separately, processed with 70% methanol in ultrasound instrument for 30 min. Also, another 100 g of dried powdered leaves were successively extracted with chloroform then with methanol at the same conditions.

Each collected extract from each process was filtered; the filtrates were dried at 40 ◦C under pressure on rotary evaporator, weighed and kept in vials.

Chromatographic and spectroscopic Manipulation of compounds from Methanol Leaves Fraction

150 g. of the methanol fraction of *H. patens* leaves (Me.L) were subjected to column chromatography with Diaion HP-20 (SUPELCO, Bellefonte, PA, USA) and eluted with gradient elution of decreasing polarity starting with 100% water and ending with 100% methanol. Fraction eluted with 50% MeOH was purified by preparative paper chromatography PPC (Whatman3MM) using butanol: acetic acid: water (BAW; 4:1:5, upper layer) as eluent [9] to yield 2 major bands, each of them was further purified by Sephadex LH-20 CC (Fluka Chemie AG, Switzerland) eluted with H_2O, resulted in isolation of two compounds (1) and (2). The identification of these compounds was performed by UV spectrophotometer, ^{1}HNMR spectroscopic analysis. On the other hand, the fraction eluted by 100% MeOH was purified by silica gel column (Silica gel 60 for CC; Merck, Darmstadt, Germany) by gradient proportions of $CHCl_3$: MeOH. Compound (3) was isolated from fraction eluted with 90% $CHCl_3$: MeOH and was analyzed by UPLC\MS\MS

In Vitro Cytotoxicity Study

SRB Cell survival assay: Potential cytotoxicity of all extracts, against liver (HepG-2) and breast (MCF-7) human tumor cell lines, was tested using SulphoRhodamine-B (SRB) method [10] in the National Cancer Institute (NCI), Cairo, Egypt. Statistical analysis: Results are expressed as mean ± S.E. The data was statistically analyzed using the Student's "t" test [11]. Doxorubicin (Pharmacia, Belgium) and Cisplatin (GlaxoSmithKlein, Egypt) cytotoxic drugs were used as references drugs.

Apparatus

UV-Visible spectrophotometer: UV-VIS double beam UVD-3500 spectrophotometer, Labomed, Inc.

Nuclear magnetic resonance (NMR) spectrometer: Bruker high performance digital FT-NMR spectrometer Avance III 400MHz, Bruker Biospin, Rheinstetten, Germany.

Ultra Performance Liguid Chromatography coupled with Ion Trap electrospray ionization mass spectrometer (UPLC/ ITMS), Ion Trap MS: MS^n mass spectra were obtained from a UPLCQ Deca XP MAX system (ThermoElectron, San Jose, USA) equipped with ESI source (electro spray voltage 4.0 kV, sheath gas: nitrogen; capillary temperature: 275 ºC) in negative and positive ionization mode. Chromatographic separations were performed by applying two elution binary gradients at a flow rate of 150 µL min^{-1}: (1) 0 to 1 min, isocratic 95% A (water/formic acid, 99.9/0.1 [v/v]), 5% B (acetonitrile/formic acid, 99.9/0.1 [v/v]); 1 to 16 min, linear from 5 to 95% B; 16 to 18 min, isocratic 95% B; 18 to 20 min, isocratic 5% B. The second binary eluent (2) was composed of ammonium acetate 50 mM buffer adjusted to pH 5 (A) and 100% acetonitrile (B) using the same elution gradient as above. The injection volume was 3.1 µL (full loop injection). Internal mass calibration of each analysis was performed by infusion of 20 µL 10 mM lithium formate in isopropanol/water, 1/1 (v/v), at a gradient time of 18 min using a diverter valve [12]

Microwave oven (CEM-MARS Microwave) A micro wave power system with user selectable power settings (0-1200 watts), open vessel for plant extraction) at 800 w with medium stirring.

Ultrasound instrument (Ultrasonic Processor UP 400 S) amplitude %: 60, cycles: 0.8.

Results

Investigation of Chemical Compounds in Methanol Leaves Fraction

Compound (1): yellow powder (15 mg), appeared as a dark purple spot on PC under UV light changed to yellow upon exposure to ammonia vapor and $AlCl_3$ spraying. Rf= 0.44 and 0.5 on PC in solvent systems BAW (4:1:5) and acetic acid (15%) respectively, suggesting flavonoid glycoside. UV data: MeOH; 259, 268, 360, NaOMe; 272, 411, NaOAc; 273, 325, 388; NaAc\ H_3BO_3; 263, 302, 380, AlCl3; 275, 304, 428; AlCl3\ HCl; 270, 304, 359, 402 indicated a flavonol type with free 7-OH, 3-OH substituted and free *O*-dihydroxyl groups at ring-B[9]. ^{1}HNMR data/DMSO revealed signals for protons at 5', 2' and 6' characteristic for quercetin. Besides; 2 anomeric protons resonate at δ 5.2 and 4.3 ppm (d, J= 7.9 and 1.7 Hz) for glucose and rhamnose moieties, respectively. Glucose exists in β-form as indicated from its large J- value but rhamnose exists in α-form as indicated from the small J-value. The upfield chemical shift of rhamnose anomeric protons proves that the sugar linkage with glucose is 1→6 (rutinoside sugar), table (1). So, compound 1 was identified as **Quercetin-3-O-rutinoside (Rutin)**. The structure was further confirmed after co-chromatography against authentic rutin. Complete acid hydrolysis for the glycoside was carried out according to the method of Harborne, 1973 [13] yielded glucose and rhamnose in the aqueous phase, identified by spotting against authentic sugars on PC (Whatmann 1 MM) with BAW (4:1:5) as eluent, using aniline phthalate spraying reagent [14] and quercetin in the ether phase confirmed by spotting against authentic on PC (Whatmann 1 MM) with BAW (4:1:5) and 50% acetic acid, Fig (1).

Compound (2): yellow powder (10 mg), Rf= 0.6 and 0.32 on PC. in solvent systems BAW (4:1:5) and acetic acid (15%) respectively. Chromatographic and spectroscopic data were similar to those of compound **(1)** except for the sugar moieties where ^{1}HNMR data showed only glucose; table (1). Compound **(2)** was identified as **Quercetin-3-O-β-D-glucoside (Isoquercetin),** Fig (1).

Compound 3: white powder (5 mg), Rf= 0.49 on silica gel TLC in solvent systems $CHCl_3$: MeOH (9:1). It appeared on TLC as a violet spot only after spraying with vanillin /sulfuric acid reagent which indicates a triterpenoidal compound. It was subjected to high resolution UPLC/ ITMS/ MS analysis, operated in both positive and negative ion modes. MS data and fragmentation pattern was compared to those in the literature and public databases such as ChemSpider, PubChem. The total ion chromatograms (TIC) of compound 3 are shown in Fig. (2-6).

The identification of compound 3 was based on determining the molecular ion from the full MS spectrum in both negative and positive modes, its chemical formula suggested by the program; Thermo X-Calibur, with calculation error less than 10 ppm and comparing them by authorized programs as ChemSpider and PubChem in attempting to get the possible structure. Then the identification was continued by determining the tandem MS fragments from MS^2 fragmentation spectrum and reviewing the literature for proper identification. Positive and Negative ion ESI-MS modes were analyzed in order to accurately investigate the compounds as possible.

$[M-H]^-$ Ion at m/z 941.5047 with a molecular ion formula $C_{48}H_{77}O_{18}$ (error: -3.760 ppm). MS^2 fragmentation in ESI-MS-MS negative mode produced fragments at m/z 795 $[M-H-Rham]^-$, m/z 633 $[M-H-Rham-hexose]^-$and m/z 457 [M-H-Rham-hexose-glucuronic acid]$^-$ which indicated the presence of 3 sugar moieties attached to each other at one position[15]. The corresponding peaks of sequential sugars losses in the +ve ion mode were also detected at m/z 797.4677 $[M-H-Rham]^-$, m/z 635.4162 $[M-H-Rham-hexose]^-$and m/z 459.3835 [M-H-Rham-hexose-glucuronic acid]$^-$

The compound also showed loss of H_2O molecules. All fragmentation peaks in both –ve and +ve ion modes are illustrated in Table (2).

The molecular ion m/z $[M-H]^-$ 457.3672 was deduced to be the aglycone, recorded the molecular ion formula ($C_{30}H_{49}O_3$; error: -0.372) indicating a triterpenoid compound. Its corresponding peak in ESI-MS^2 fragmentation spectrum in positive mode after sugars cleavage had appeared the aglycone peak at *m/z* $[M+H]^+$ 459.3835, followed by peaks at *m/z* 441.3726 [aglycone -18], *m/z* 423.3619 [441.3726-18] and *m/z* 405.3517 [423.3618-18] which indicated the

consecutive losses of H_2O molecules. These data were in full matching with previous literature [16], revealing that the aglycone part most probably is soyasapogenol Bb. So the whole compound could be identified as the triterpenoid oligosaccharide (triterpenoid glycosides) saponin of the oleanane type; **Soyasaponin Bb**; 22,24-dihydroxyolean-12-en-3-yl-rhamnopyranosyl-(1->2)-beta-D-galactopyranosyl-(1->2)-beta glucouronic acid. The structure is shown in Fig. (1). The suggested cleavage pattern of compound 3 may be as provided in Fig. (7).

It's belonging to the diverse bioactive group of soyasaponins primarily found in legumes, especially Soy bean [17]. Soyasaponins in group B are known as monodesmosidic saponins; which means that the aglycone of this group possesses only one site of glycosylation which is C-3 for soyasapogenol B [15].

Several extraction techniques were investigated in order to increase the productivity, decrease excess solvents hazards, and improve the yield and the quality of the extracted products. The percentage yields of all samples were listed

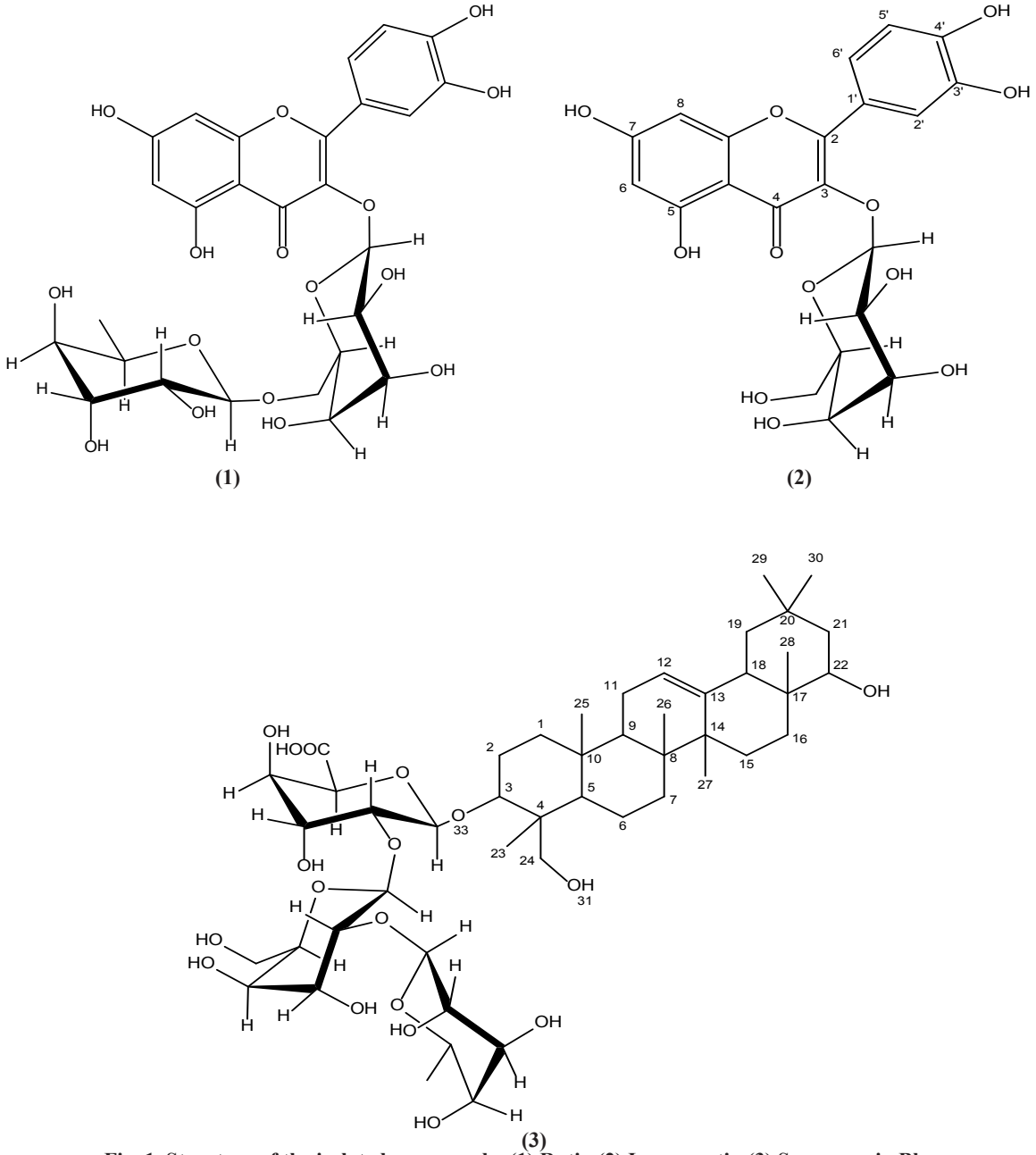

Fig. 1. Structure of the isolated compounds; (1) Rutin (2) Isoquercetin (3) Soyasaponin Bb.

TABLE 1. ^{1}HNMR data for compound (1) and (2).

Proton No.	δ 1H (ppm) (J in Hz)	
	Compound (1) Rutin	Compound (2) Isoquercetin
6	6.14 (S, 2H)	6.2 (S, 1H)
8	6.33 (S, 2H)	6.4 (S, 1H)
5’	6.81, 6.83 (d, 1H, J=8.5)	6.8 (d, 1H, J=9)
2’	7.52 (1H, 8.5)	7.58 (1H, q, J=2.5, 8.5)
6`	7.63-7.65 (1H, d, J=8.5)	7.7 (1H, d, J= 2.5)
1``	5.3 (d, J=7.4, 1H)	5.4 (d, J= 6.6, 1H)
2``	3.1 – 3.7	3.2 – 3.8
3``		
4``		
5``		
6``		
1```	4.4 (d, 1H)	-------
2```	3.1-3.7	
3```		
4```		
5```		
CH_3	0.99-1.01 (3H, d, J= 6.5)	

TABLE 2. UPLC-ESI-MS-MS fragmentation ions of compound 3 in both negative and positive ion modes.

Negative ion mode (m/z)		Positive ion mode (m/z)	
Prominent MS fragments	**Molecular ion formula**	**Prominent MS fragments**	**Molecular ion formula**
941 $[M-H]^-$	$C_{48}H_{77}O_{18}$ (Error: -3.760 ppm)	**943** $[M+H]^+$	$C_{48}H_{79}O_{18}$ (Error: 0.742 ppm)
923	$C_{48}H_{75}O_{17}$ $[M-H-H_2O]$	925	$C_{48}H_{75}O_{17}$ $(M-H-H_2O)$
795	$C_{42}H_{67}O_{14}$ (M-H-rhm)	**797 (100%)**	$C_{42}H_{67}O_{14}$ (M+H-rhm)
(100%) 733	$C_{41}H_{65}O_{11}$ (879-rhm)	---	--
633	$C_{36}H_{57\ O9}$ (795-hexose)	**635**	$C_{36}H_{57}O_9$ (M+H-rhm-hexose)
615	$C_{36}H_{55}O_8$ (923-rhm-hexose)	617	$C_{36}H_{55}O_8$ (925-rhm-hexose)
457	$C_{30}H_{49}O_3$ (error: -0.372) (M-H-rhm- hexose -glucuronic acid)	**459**	$C_{30}H_{49}O_3$ (M+H-rhm- hexose -glucuronic acid)

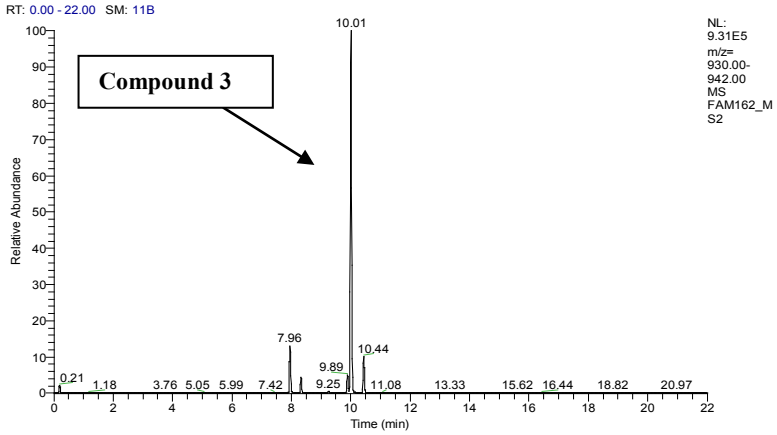

Fig. 2. BPC of compound 3 in ESI-MS –ve mode.

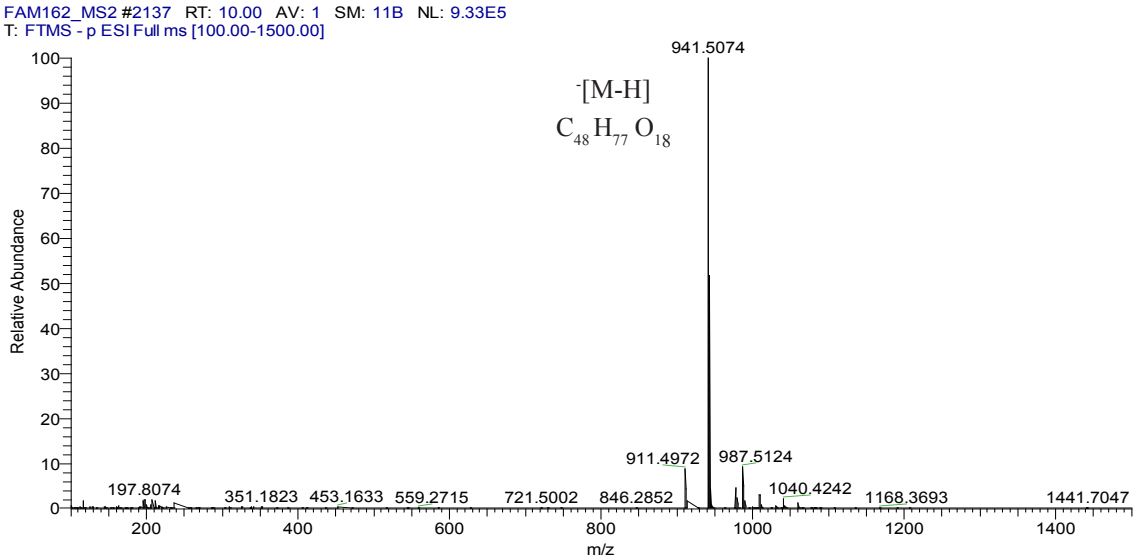

Fig. 3. Full MS spectrum of compound 3 at Rt. 10 min. in the negative ion mode $[M-H]^-$.

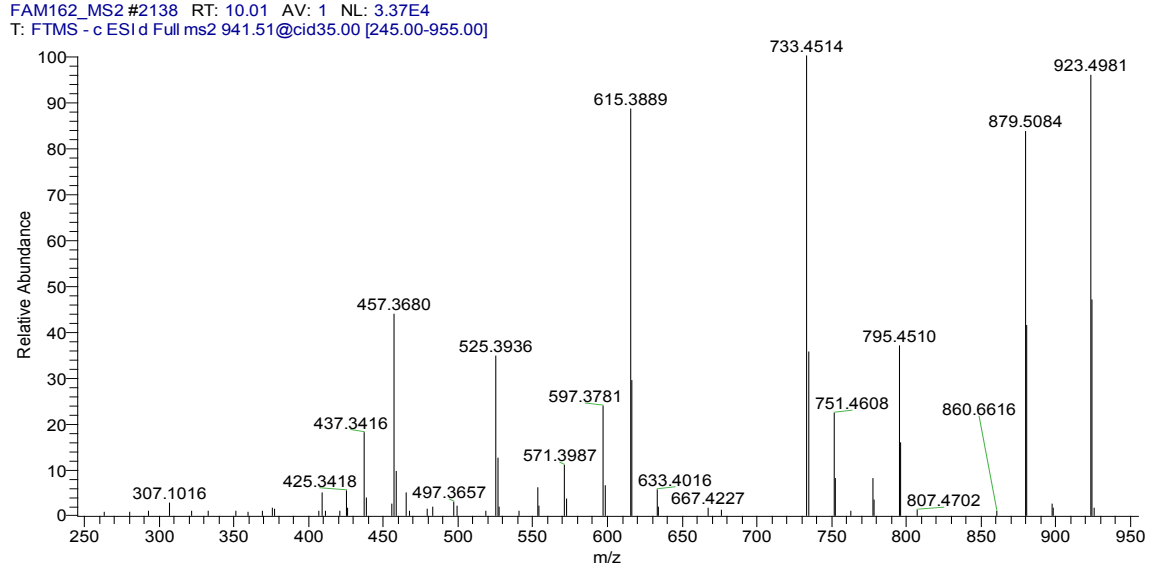

Fig. 4. MS^2 spectrum of compound 3 at Rt. 10 min. and its fragmentation peaks in the negative ion mode.

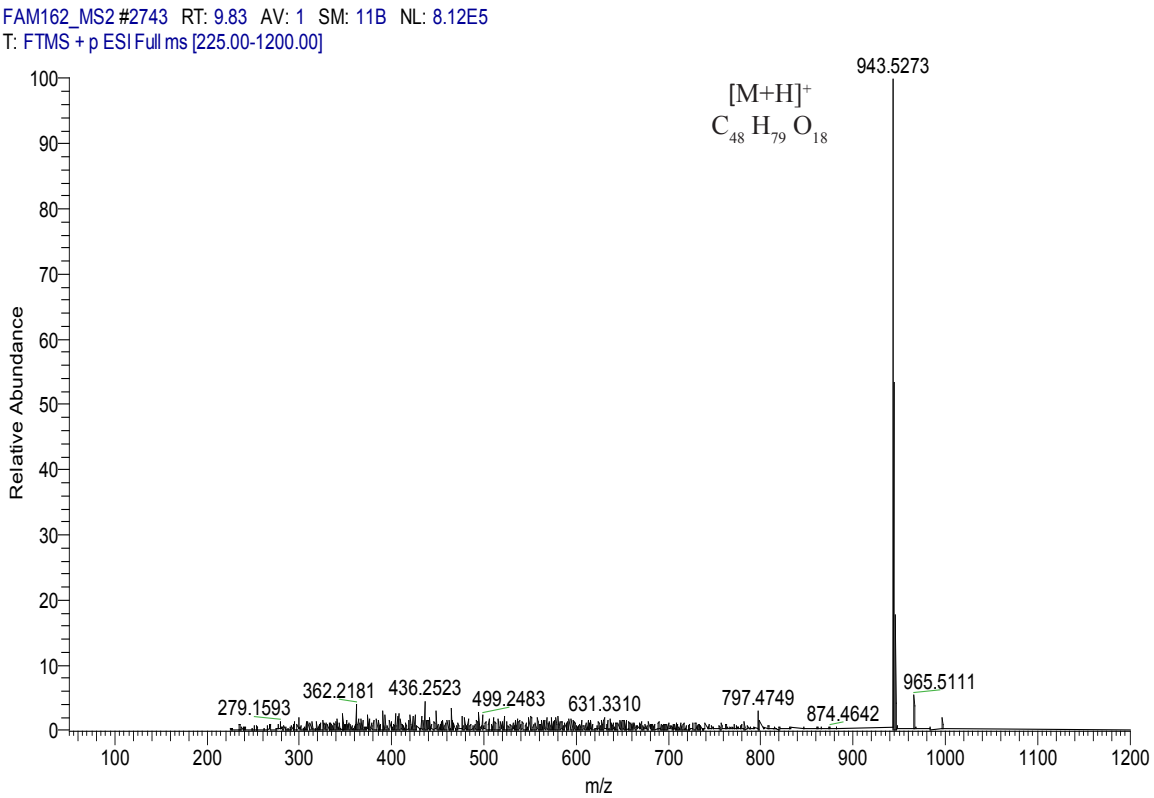

Fig. 5. Full MS spectrum of compound 3 at Rt. 9.83 min. in the positive ion mode $[M+H]^{-}$.

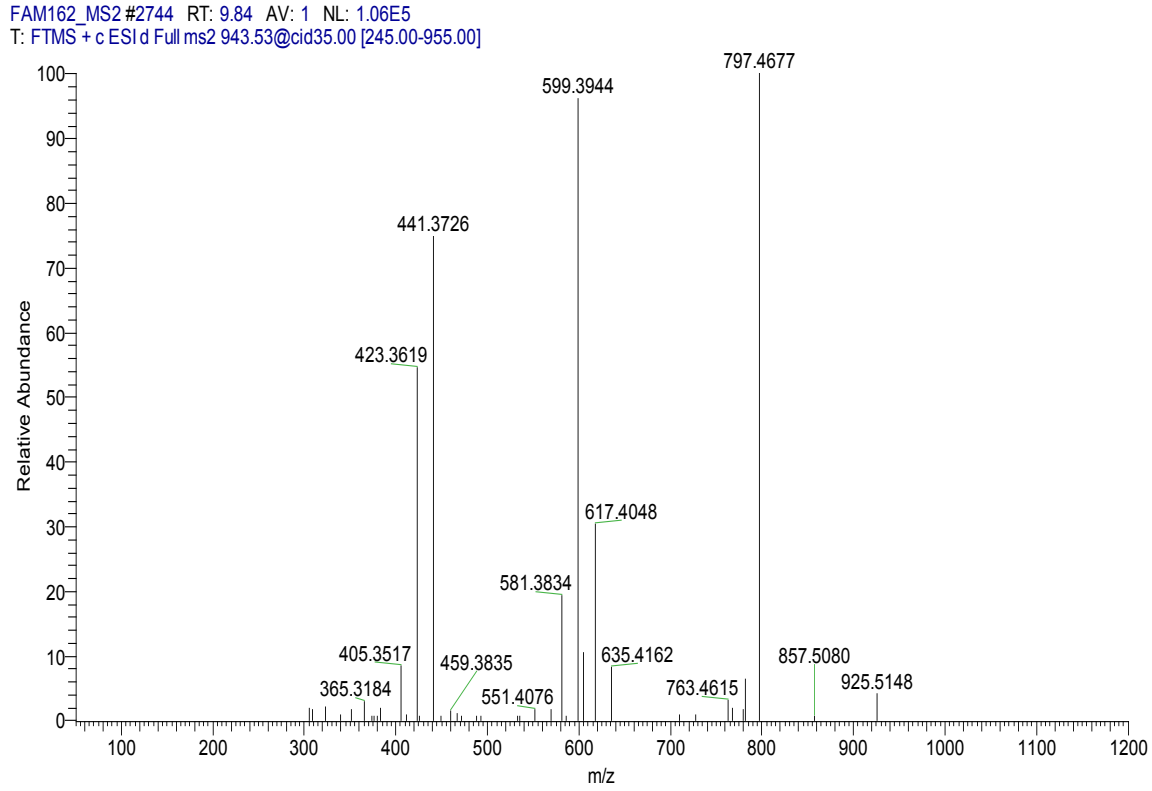

Fig. 6. MS^2 spectrum of compound 3 at Rt. 9.84 min. and its fragmentation peaks in the positive ion mode.

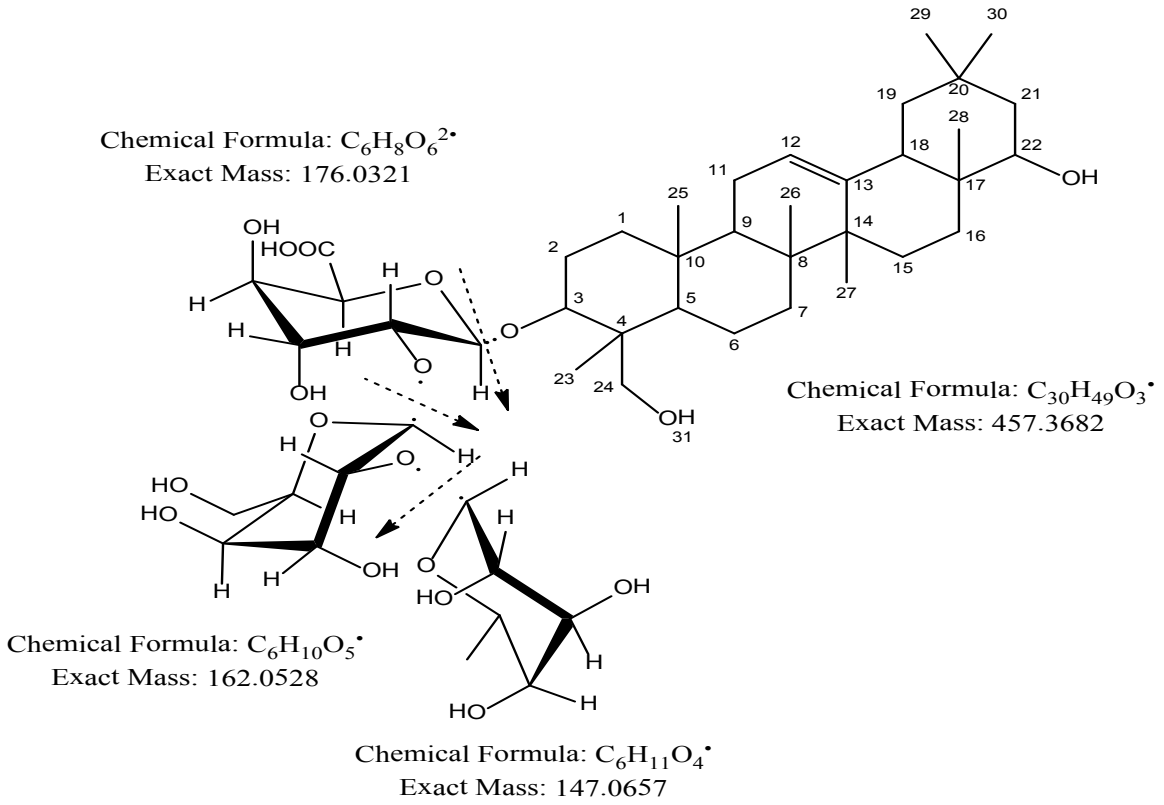

Fig. 7. The possible fragmentation pattern of compound 3, Soyasaponin Bb.

in table (3).

Table (3) showed that the highest percentage yield of the CL resulted from that extracted by MAE-30 min followed by CP-30 min., while UAE-30 min was the least method yielded an extract. After 6 times of CP, it gave the yield of 25.6% crude leaf extract, barely reached the same level of MAE yield after 30 min.

Also, the highest percentage yield of the CF resulted from that extracted by MAE-30 min followed by UAE-30 min., while CP-30 min was the least method yielded an extract. CP after 6 times of extraction gave the yield of 38% CF that even couldn't reach the same level of MAE yield after 30 min. For the successive fractions, no observable differences between MAE and UAE in Chl.L or Me.L fractions, noting that chloroform extraction gave lower yield from MAE than UAE. This is a reasonable result as MAE is considered a selective method that prefers polar solvents with high dielectric constant [18].

The previous comparative experiment revealed that CP is no longer an effective procedure for extraction as it's largely time consuming, in addition to the very high solvent amounts that are needed to elevate the extraction yield. These

TABLE 3. % yield of CL and CF extracts of H. patens from different methods of extraction.

Extract	% yield from different methods of extraction			
	CP (After 30 m.)	**CP (After 6 times.)**	**MAE (After 30 m.)**	**UAE (After 30 m.)**
CL	13 %	25.6%	**29.26 %**	10.4 %
CF	12.2 %	38%	**43%**	13.67 %
Chl.L	ND	ND	2.17 %	**3.5 %**
Me.L	ND	ND	6 %	**6.5 %**

CP: Cold Percolation.
MAE: Microwave Assisted Extraction.
UAE: Ultrasonic Assisted Extraction.
ND: not detected

large volumes of organic solvents need proper management of the chemical wastes. This is a major problem as compared to UAE and MAE, which is known as the "Green methods" [19]. Moreover, it's largely time consuming.

The advantages of MAE technique are that it reduces extraction time and solvent volume as compared to the other conventional methods (maceration or Soxhlet extraction). Also, improved recoveries of components and reproducibility were observed. But it should be used with caution of using proper conditions to avoid thermal degradation [20]. UAE procedure is considered a simple and relatively cheap technology that can be used for phytochemical extraction in both small and large scales. Its benefits are mainly in reducing the extraction time and solvent consumption.

In vitro cytotoxic study

Investigating the potential antiproliferative effect of the plant extracts against cancerous cell lines revealed that all extracts have shown potent efficiency in inhibiting the growth of cancerous cell lines in a dose dependent manner. The action was higher against MCF-7 than HepG-2 for CF, CL and Chl.L, while Me.L was more active against HepG-2. CL showed no activity against HepG-2 at the tested concentrations. IC_{50} values for all samples as well as the reference drugs are listed in table (4), Fig. (8-11).

It is worthy to state that; The American National Cancer Institute guidelines (NCI, USA) has put the criteria for the antiproliferation activity for any crude extract; the potent cytototoxic extract is the one with IC_{50} less than 30 µg/mL after 24 h exposure time with cells [21].

The overall results support that *H. patens* leaves and flowers have great potential to be efficient agents in treating breast and liver cancer.

Discussion

Our chemical investigation for leaves and flowers of *H. patens* Jacq had been indicated the presence of numerous chemical groups; high

TABLE 4. IC_{50} of different samples against HEPG-2 and MCF7.

Tested cancerous cell lines	IC_{50} (µg/mL)					
	CF	CL	Chl.L	Me.L	Doxorubicin	Cisplatin
HEPG-2	47	>100	30	**44.4**	5.87	9.83
MCF7	**23.8**	**25.5**	**17.7**	64	3.8	0.6

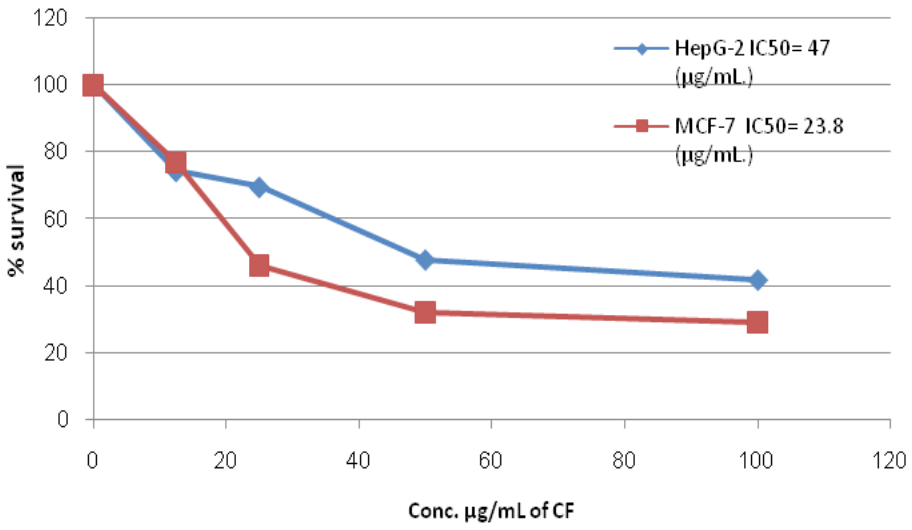

Fig. 8. Cytotoxic activity of CF against HEPG-2 and MCF-7cell lines.

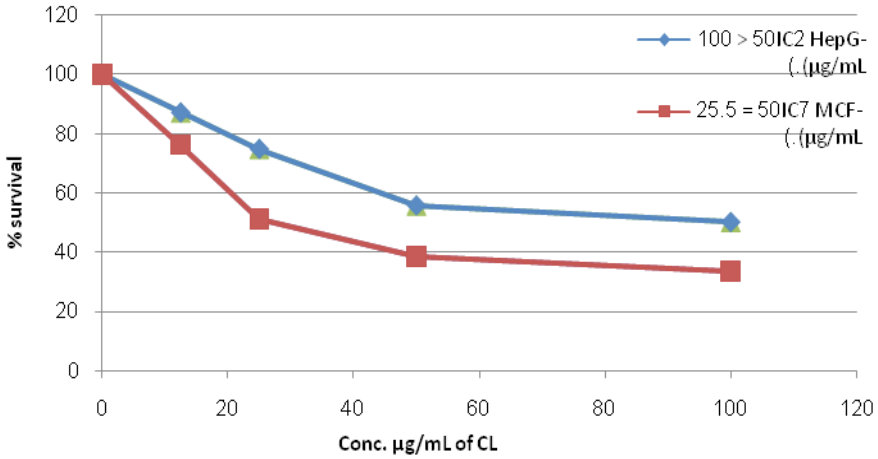

Fig. 9. Cytotoxic activity of CL against HEPG-2 and MCF-7cell lines.

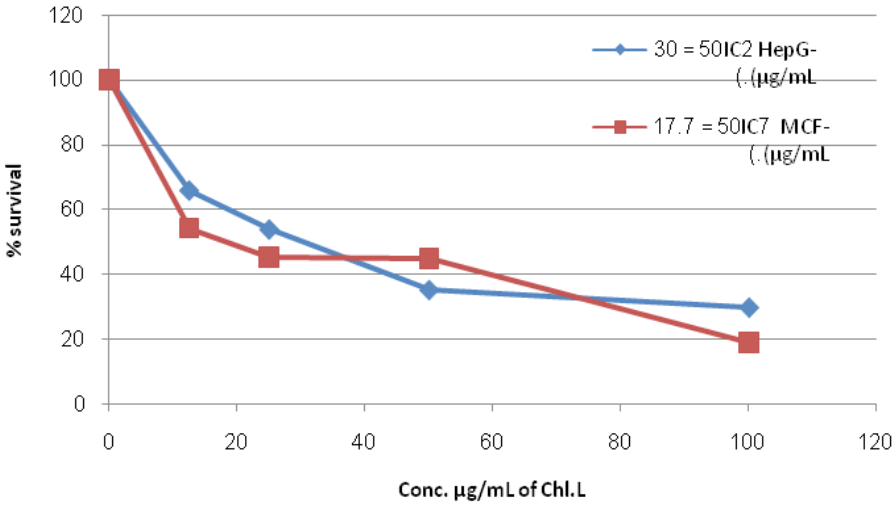

Fig. 10. Cytotoxic activity of Chl.L against HEPG-2 and MCF-7cell lines.

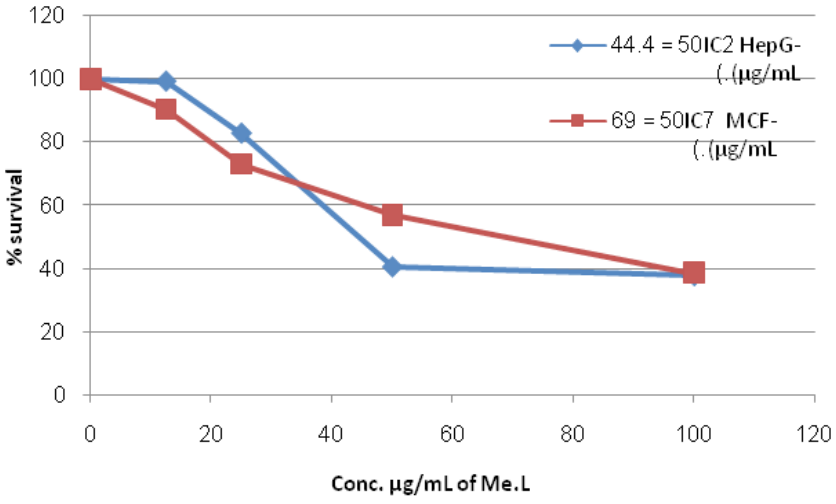

Fig. 11. Cytotoxic activity of Me.L against HEPG-2 and MCF-7cell lines.

amount of carbohydrates, glycosides, flavonoids, phenolics, sterols, triterpenes, alkaloids and moderate amount of saponins. In this study, continuous chromatographic analysis was performed in order to isolate chemical compounds. That resulted in the isolation and identification of 2 flavonoid glycosides; rutin and isoquercetin in addition to a triterpene glycoside; soyasaponin Bb. The later was isolated from the plant for the first time. Examining the cytotoxic activity of *H. patens* leaves and flowers with SRB assay had shown promising effect against liver and breast cancer cells. This may be attributed to the presence of high amounts of polyphenolic compounds. Rutin, the abundant flavonol present in various plants demonstrated anticancer effects against different cell lines. It acted on reducing tumor size of HL-60 Leukemic cells [22]. It also arrested the cell cycle and induced apoptosis of colorectal cancer [23]. It also has a cytotoxic effect against HTC hepatic carcinoma cells [24]. Generally, quercetin and its derivatives are known to be efficient cytotoxic agents [25]. Maiyo et al (2016), [26] had tested the cytotoxicity of quercetin-3-O-glucoside against liver hepatocellular carcinoma (HepG-2) and colon carcinoma (Caco-2) and they had found that it exerted potent *in vitro* cytotoxic and apoptotic effect on the cancerous cell lines. On the other hand, Soyasaponin B found to possess anti-cancer properties by modulating the cell cycle and inducing apoptosis. Also, a mixture of sayasaponin fraction gave cytotoxic effect on Hela cells via apoposis [27].

Conclusion

At the end of our work it could be concluded that the chemical constituents isolated from natural sources could be efficient treating agents for various ailments. More studies should be continued on that plant to explore more bioactive agents.

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References

1. Martins D and Nunez C V. Secondary Metabolites from Rubiaceae Species. *Molecules*, **20** (7), 13422-13495 (2015)

2. Aquino R, Ciavatta M L, De Simone F, Pizza C A. A flavanone glycoside from *Hamelia patens*. *Phytochemistry;* **29**(7), 2358-2360 (1990).

3. Paniagua-Vega D, Cerda-García-Rojas CM, Ponce-Noyola T, Ramos-Valdivia AC. A new monoterpenoid oxindole alkaloid from *Hamelia patens* micropropagated plantlets. *Nat Prod Commun;* **7**(11), 1441-1444 (2012).

4. Jiménez-Suárez V, Nieto-Camacho A, Jiménez Estrada M and Alvarado Sánchez B. Anti-inflammatory, free radical scavenging and alpha-glucosidase inhibitory activities of *Hamelia patens* and its chemical constituents, *Pharmaceut. Biol.*, **5**4 (9), 1822-1830 (2016).

5. Paz J E, Contreras C R, Munguíab A R, Aguilar C N, Carrillo M L and Inungaray M C. Phenolic content and antibacterial activity of extracts of *Hamelia patens* obtained by different extraction methods. *Braz J Microbiol.* **49**(3), 656-661 (2018).

6. Camporese A, Balick M J, Arvigo R, Esposito R G, Morsellino N, De Simone F, and Tubaro A. Screening of antibacterial activity of medicinal plants from Belize (Central America). *J. Ethnopharmacol.*; **87**(1), 103-107 (2003).

7. Khandelwal S, Sharma P, Singh T and Vijayvergia R. Quantitative estimation and comparative study of primary metabolites of some medicinal plants. *Curr. Pharmaceut. Res.*, **2**, 378- 381 (2011).

8. Abubacker M N, Sathya C and Prabakaran R. *In vitro* Antifungal Potentials of *Hamelia patens* Jacq. (Rubiaceae) Aqueous Extracts of leaves, flowers and fruits. *Biosci. Biotech. Res. Asia;* **10** (2), 699-704 (2013).

9. Mabry T J, Markham K R and Thomas M B. The Systematic Identification of Flavonoids. Springer Verlag, Berlin. (1970).

10. Skehan P, Storeng R, Scudiero D, Monks A, McMahon J, Vistica D, Warren J T, Bokesch H, Kenney S and Boyd M R. New colourimetric cytotoxicity assay for anticancer drug screening. *J Nat Canc Inst,* **82**, 1107-1112 (1990).

11. Snedecor W G and Cochran G W. Statistical Methods, 7th Edn. Ames, Iowa State University Press, USA. (1980).

12. Farag M A, Wessjohann L A. Metabolome Classification of Commercial *Hypericum perforatum* (St. John'sWort) Preparations via UPLC qTOF MS and Chemometrics. *Planta Med*.**78**, 488-496. (2012).

13. Harborne J B. Phytochemical Methods. Chapman and Hall, London, P. 215. (1973).

14. Partridge S M. Aniline hydrogen phthalate as a spraying reagent for chromatography of sugars. *Nature*, **164**, 443 (1949).

15. Shiraiwa M, Harada K, Okubo K. Composition and structure of group B saponin in soybean seed. *Agric. Biol. Chem*., **55**, 911-917 (1991).

16. Tsuno Y, Fujimatsu T, Endo K, Sugiyama A and Yazaki K. Soyasaponins: A New Class of Root Exudates in Soybean (*Glycine max*), *Plant and Cell Physiology*, **59** (2), 366–375 (2017).

17. Rao A V, Sung M K. Saponins as anticarcinogens. *J. Nutr.,* 125, 171s-174s (1995).

18. Handa S S, Khanuja S P S, Longo G and Rakesh D D. *Extraction Technologies for Medicinal and Aromatic Plants* (1st edn), no. 66. Italy: United Nations Industrial Development Organization and the International Centre for Science and High Technology (2008).

19. Dhanani T, Singh R, Shah S, Kumari P, Kumar S. Comparison of green extraction methods with conventional extraction method for extract yield, L-DOPA concentration and antioxidant activity of *Mucuna pruriens* seed. *Green Chemistry Letters and Reviews*, **8**, 2, 43-48 (2015).

20. Kaufmann B and Christen P. Recent extraction techniques for natural products: microwave-assisted extraction and pressurized solvent extraction. *Phytochem. Ana.l* **13**, 105-113 (2002).

21. Suffness M and Pezzuto J M. Assays related to cancer drug discovery. In: Hostettmann K (ed). *Methods in Plant Biochemistry: Assays for Bioactivity,* Vol. 6, Academic Press, London. P. 71-133 (1990).

22. Lin J P, Yang J S, Lin J J, Lai KC, Lu H F, Ma C Y, et al. Rutin inhibits human leukemia tumor growth in a murine xenograft model *in vivo*. *Environ. Toxicol.*, **27** (8), 480-484 (2012).

23. Araújo J R, Gonçalves P and Martel F. Chemopreventive effect of dietary polyphenols in colorectal cancer cell lines. *Nutr. Res*. **31** (2), 77-87 (2011).

24. Marcarini C J, Tsuboy F M S, Luiz C R, Ribeiro R L, Hoffmann-Campo B C, Mantovani S M. Investigation of cytotoxic, apoptosis-inducing, genotoxic and protective effects of the flavonoid rutin in HTC hepatic cells. *Exp. Toxicol. Pathol.,* **63** (5), 459-465 (2011).

25. Dell'Albani P, Di Marco B, Grasso S, Rocco C and Foti M C. Quercetin derivatives as potent inducers of selective cytotoxicity in glioma cells. *European Journal of Pharmaceutical Sciences*. **101**, 56-65 (2017).

26. Maiyo F C, Moodley R, Singh M. Cytotoxicity, Antioxidant and Apoptosis Studies of Quercetin-3-O Glucoside and 4-(β-D-Glucopyranosyl-1→4-α-L-Rhamnopyranosyloxy)-Benzyl Isothiocyanate from *Moringa oleifera*. *Anticancer Agents Med Chem*., **16**(5), 648-56 (2016).

27. Xiao J X, Huang G Q, Zhu C P, Ren D D and Zhang S H. Morphological study on apoptosis Hela cells induced by soyasaponins. *Toxicology in Vitro*. **21** (5), 820-826 (2007).

التوصيف الكيميائي للمركبات المفصولة من نبات هاميليا باتنس والتحقيق في نشاطه السام للخلايا السرطانية

مى عبد النبى إسماعيل مأمون[١]، سلمى أحمد محمود الساوى[١]، حماية محمد مطاوع[١]، مصطفى إبراهيم فكرى[٢]، مصطفى على عبد القوى[٢]

[١]قسم العقاقير- شعبة بحوث الصناعات الصيدلية و الدوائية - المركز القومى للبحوث -القاهرة - مصر.

[٢]قسم العقاقير- كلية الصيدلة - جامعة القاهرة - القاهرة - مصر.

يهدف العمل الحالي إلى استكشاف المركبات النباتية من نبات هاميليا باتنس المزروع في مصر وعزل المركبات الكيميائية بتقنيات الكروماتوغرافي وتحديد هذه المركبات بطرق التحليل التحليلي المختلفة. كما تم التحقيق في التأثير السام للخلايا المستخلصة من المستخلصات الخام من الأزهار والأوراق وكذلك الأجزاء المتعاقبة غير القطبية والقطبية المتتالية من الأوراق.

نتج عن التحليل الكروماتوجرافي لمستخلص الميثانول لاوراق هاميليا باتنس القيام بالفصل والتعرف، بالتحليل الطيفي، على مركبى الروتين (1)، و الأيزوكويرسيتين (2) بالاضافة الى صوياصلبونين 3) Bb) و الذي تم عزله لأول مرة من أوراق النبات وتم التعرف عليه بواسطة تقنية UPLC/ITMS/MS. و قد تم إجراء اختبار السمية الخلوية ضد خلايا الكبد HepG-2 والثدي السرطانيةMCF-7 لمستخلصات الزهور الخام (CF)، والأوراق الخام (CL)، وكذلك مستخلصى الكلوروفورم (Chl.L) والميثانول (Me.L) من الأوراق و ذلك باستخدام تقنية SRB.

و قد أظهرت النتائج فعالية هذه المستخلصات ضد نمو الخلايا السرطانية حيث كانت التركيز المتسبب فى قتل نصف عدد خلايا HEPG-2 لـ CF و Chl.L و 47 = Me.L IC50 و 30 و 44.4 ميكروغرام / مل على التوالي. من ناحية أخرى ، كان CF و CL و Chl.L و Me.L تأثير قوي ضد MCF-7 ووجد ان IC50 = 23.8 و 25.5 و 17.7 و 64 ميكروغرام / مل على التوالي.

كما تم فحص دراسة مقارنة لاستخدام الطريقة العادية مقابل الطرق الخضراء للاستخلاص و قد وجد ان طريقة الاستخلاص بالميكروويف هو من احسن الطرق التى تعطى اكبر كمية مستخلصات، توفر الوقت و توفر استخدام المذيبات العضوية.

في نهاية عملنا يمكن أن نستنتج أن المكونات الكيميائية المعزولة من المصادر الطبيعية يمكن أن تكون عوامل علاج فعالة للعديد من الأمراض. يجب مواصلة المزيد من الدراسات على هذا المصنع لاستكشاف المزيد من العوامل النشطة بيولوجيًا.